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An investigation into the effects of in vitro dilution with different colloid resuscitation fluids on clot microstructure formation

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Abstract

Background: Balancing the beneficial effects of resuscitation fluids against their detrimental effect on hemostasis is an important clinical issue. We aim to compare the *in vitro* effects of 3 different colloid resuscitation fluids (4.5% albumin, hydroxyethyl starch (Voluven® 6%) and gelatin (Geloplasma®)) on clot microstructure formation using a novel viscoelastic technique, the Gel Point. This novel haemorheological technique measures the biophysical properties of the clot and provides an assessment of clot microstructure from its viscoelastic properties. Importantly, in contrast to many assays in routine clinical use, the measurement is performed using unadulterated whole blood, in a near patient setting and provides rapid assessment of coagulation. We hypothesized that different colloids will have a lesser or greater detrimental effect on clot microstructure formation when compared against each other.

Methods: Healthy volunteers were recruited into the study (n=104) and a 20ml sample of whole blood obtained. Each volunteer was assigned to one of the three fluids and the sample was diluted to one of five different dilutions (baseline, 10, 20, 40 and 60%). The blood was tested using the Gel Point technique which measures clot mechanical strength (G'_{GP}) and quantifies clot microstructure (d_f) at the incipient stages of fibrin formation.

Results: d_f and G'_{GP} decrease with progressive dilution for all three fluids. A significant reduction in d_f from baseline was recorded at dilutions of: 20% for albumin ($P < 0.0001$); 40% for starch ($P < 0.0001$) and 60% for gelatin ($P < 0.0001$). We also observed significant differences, in terms of d_f , when comparing the different types of colloid ($P < 0.0001$). We found that albumin dilution produced the largest changes in clot microstructure, providing the lowest values of d_f ($=1.41 \pm 0.061$ at 60% dilution) compared to starch (1.52 ± 0.081) and gelatin (1.58 ± 0.063).

Conclusions: We show that dilution with all three fluids has a significant effect on coagulation at even relatively low dilution volumes (20 and 40%). Furthermore, we quantify, using a novel viscoelastic technique, how the physiochemical properties of the three colloids exert individual changes on clot microstructure.

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Introduction

Anesthetists and critical care specialists use a variety of intravenous fluids to correct volume depletion in the vascular space, in order to maintain normal tissue and organ perfusion.^{1,2} There are two main categories of fluids used, crystalloids or colloidal suspensions. Crystalloids are mainly recognized to have an adverse effect on coagulation, due to their purely extrinsic dilutional effect.^{3,4} In comparison colloids in addition to any dilutional effect, have an intrinsic effect on coagulation due to the physiochemical properties of the colloids.³⁻⁷ The intrinsic effects of the colloids can have a pronounced detrimental effect on coagulation when compared to crystalloids and are associated with increased blood loss and unfavorable outcomes.⁸ Despite this colloids are still used routinely, as they produce a quicker and more pronounced physiological effect in maintaining tissue perfusion.^{1,2}

Previous studies have attempted to investigate the effects of dilution with different colloids on coagulation by using viscoelastic techniques including Thromboelastography (Haemoscope Corporation, Niles, IL).^{3-5,11} These studies have shown that colloids have a detrimental and varying effect on coagulation. However, there is continued debate and conflicting evidence on which colloid has the most detrimental effect on coagulation, and deciding on what colloid to use, when/if to use it and in what quantities still remains controversial.⁸

Several studies have highlighted the importance of fibrinogen in correcting the effects of dilution on coagulation.^{9,10} Recent advances in our understanding of viscoelastic changes in coagulation have resulted in the development of new techniques for the analysis of clot characteristics, including clot microstructure.¹² A previous study using

a new functional biomarker of clot microstructure, has identified how isolated dilution with a crystalloid changes clot microstructural organization and strength.¹² This novel technique uses whole blood and measures the formation of the Gel Point (GP) which provides values of the elasticity of the clot (G'_{GP}) and a numerical description of the clots microstructure (d_f).¹³⁻¹⁵ The d_f is a quantitative measure of *clot microstructure*, a decrease in d_f corresponding to more permeable, less branched polymerized weaker fibrin structure.¹⁶

We hypothesize that dilution with colloid resuscitation fluids have a detrimental effect on clot microstructure formation as measured by d_f . Additionally we aim to investigate whether different colloids will have a lesser or greater detrimental effect on clot microstructure formation when compared against each other. In this study we investigate three commonly used colloids to determine which has the most detrimental effect on clot microstructure in a purely *in vitro* model of fixed volume dilution. The three colloids used in this study include a naturally occurring colloid 4.5% albumin and two synthetic colloids: a hydroxyethyl starch based polymer suspension (Voluven® 6%) and a gelatin based polymer suspension (Geloplasma®).

Materials and methods

Study Design The study was approved by local Research Ethics Committee (REC 07/WMW/136). Written informed consent was obtained from all subjects before enrolment. Healthy volunteers were included if they met the following criteria: 18 years and above, no history of an acute or chronic condition known to effect coagulation (e.g. cancer, hepatic and/or renal dysfunction) and no personal or family history of bleeding or thromboembolic disorders, or have taken any medication known to effect coagulation, with all participants having a normal full blood count and clotting profile. Subjects were excluded if currently undergoing any anti-platelet or anti-coagulation treatment. The three different fluids used in this study were a gelatin based colloid (Geloplasma, anhydrous gelatin/15g, Fresenius Kabi Ltd. Cheshire, UK; BN: 13GIL272), a commonly used hydroxyethyl starch, (Voluven® 6%, starch 130/0.4, Fresenius Kabi Ltd. Cheshire, UK; BN:13EML053) and a naturally occurring colloid (4.5% human albumin, Zenlab® 4.5%, Bio Products Laboratory Ltd. Herts, UK; BN: ADAN0425).

Blood Sampling

Prior to taking the sample, each volunteer was assigned to one of the three different colloidal fluids, they were then assigned to one of the four dilutions tested in this study. The dilutions investigated for each fluid were 10% (n=8), 20% (n=8), 40% (n=8) and 60% (n=8). An additional 8 volunteers were recruited for a 0% dilution reference range. For each volunteer a 20ml sample of blood was obtained from the antecubital vein via an 18 gauge needle. The sample was added to a specified volume of their designated fluid and gently inverted three times to aid mixing. The diluted blood was divided into two aliquots. The first (7ml) was immediately transferred to the measuring geometry of

a Rheometer for viscoelastic analysis. The second aliquot ($\approx 9\text{ml}$) was used to obtain a coagulation screen and full blood count (FBC). The limitations of the study include the inability to run all 5 dilutions (0%, 10%, 20%, 40% and 60%) for all three colloids for each volunteer at one sampling point. The GP technique uses whole blood limiting the number of experiments that can be run at any one time by the availability of machines.

Rheometric Measurements: The Gel Point

A 6.6 ml aliquot of diluted blood was immediately loaded into a TA Instruments AR-G2 controlled-stress rheometer (TA Instruments, New Castle, DE, USA) at 37°C ($\pm 0.1^\circ\text{C}$). All measurements were made on aliquots of blood using similar measuring geometries with identical measuring surfaces and surface preparation procedures. The measuring geometry used was a double-gap concentric cylinder (TA Instruments, New Castle, DE, USA) (see Figure 1). Once loaded the experiment was started, the time from venipuncture to starting the experiment was timed and was always $<60\text{seconds}$.

The GP measurement involves performing oscillatory deformations over a range of frequencies, called small amplitude oscillatory shear measurements. The measurement uses four different frequencies (2Hz, 0.93Hz, 0.43Hz and 0.2Hz) (see Figure 2) with a peak stress amplitude of 0.03Pa. The small amplitude oscillatory shear measurements provide assessment of the *visco-elastic* properties of the material. The small amplitude oscillatory shear experiments measure: the shear elastic modulus, G' (a measure of elastic component of the sample); the loss modulus, G'' (a measure of viscous component of the sample); and phase angle, δ (where: $\tan \delta = G''/G'$). δ represent the ratio of the viscous and elastic components of the blood and has a range of 0 to 90° where 90° identifies a purely viscous response and 0° identifies a purely elastic

response, with all values in-between being visco-elastic. We measure δ at 4 different frequencies sequentially (with time) to record how δ changes as the blood clots. In elasticoviscous fluids the higher the frequency the lower the value of δ that will be recorded at the same point in time, conversely in viscoelastic solids the higher the frequency the higher the value of δ (see Figure 2). Therefore by measuring multiple frequencies during a GP measurement, the transition of the blood from a elasticoviscous fluid to a viscoelastic solid can be identified at the point where δ is independent of frequency, where the material changes from a liquid to a solid.¹⁷ This point is called the GP which in coagulating blood corresponds to the formation of the incipient clot.¹³ The incipient clot defines the point where fibrin network becomes sample spanning and establishes sufficient connectivity to confer elastic solid-like properties required for its haemostatic function.¹³

It is known that the viscoelastic properties at the GP are directly linked to the structural properties of the system which can be expressed in terms of fractal dimension, d_f .^{13, 18} Fractal dimension is a tool commonly used to quantify complex structures. The greater the value of d_f , the more compact/dense is the network structure, whereas low values of d_f correspond to more open/permeable networks. At the GP, fractal dimension, d_f , is related to the stress relaxation exponent θ which can be obtained from the value of δ at the Gel Point where $\delta_{GP} = \theta\pi/2$. The relationship between d_f and θ is described by the equation: $d_f = (D + 2)(2 - \theta)/2(\theta - D)$ where D is the space dimension ($D = 3$ herein).^{12-15,18}

Mass vs d_f :

In conjunction with the gel point measurements we also provide a previously published computational simulation.^{16,19,20} This simulation was used alongside the experimental data collected from the gel point measurements, to help illustrate how any changes in incipient clot microstructure (d_f) will relate to changes in the mass of the clot. Previous studies using light scattering and microscopy have established that incipient fibrin clots have fractal properties, where the mass, M , is related to d_f by the following power law equation [$M \approx \epsilon^{d_f}$, where ϵ is some length scale value in the range 100nm to 10 μ m].^{20,21} This non-linear relationship is presented in Figure 3.

Laboratory Markers

Full blood Count: A 4 ml aliquot of blood was collected into plastic, full-draw dipotassium EDTA Vacuettes (Greiner Bio-One, Stonehouse, UK Ref: 454286) for FBC analysis and was analyzed using a Sysmex XE 2100 (Sysmex UK, Milton Keynes, UK) automated hematology analyzer within two hours of collection. Fibrinogen concentration was verified against the 2nd International Fibrinogen Standard Version 4 (NIBSC code 96-612). All reagents were obtained from Siemens, (Frimley, UK). The analyzer was calibrated according to manufacturer's instructions.

Coagulation Screen: An additional 4.5ml was transferred immediately into citrated siliconized glass Vacutainers (0.109M) (Becton-Dickinson, Plymouth, UK Ref: 367691) for routine coagulation studies. PT, APTT and Clauss fibrinogen were measured using a Sysmex CA1500 analyzer within two hours of collection. All reagents were obtained from Siemens, (Frimley, UK).

Statistical analysis

Statistical analysis was performed using Minitab® version 16 software (Havertown, PA) and GRAPHPAD PRISM® version 6.0 (GraphPad software Inc., La Jolla, CA, USA). We powered this study for the main effect that each of the different fluids would produce a significant change in the d_f measurement. using data from a previous study where the d_f at 0% dilution was 1.73 ± 0.035 , and expecting a decrease in d_f of around 0.06 as dilution is increased.¹² Assuming an α of 0.05 and a power of 0.90, we calculated that 8 subjects would be required for each studied dilution, with a total of 4 different dilutions and 3 different fluids (with an additional 8 for a 0% dilution) giving a total of 104 participants.

All results included are reported as mean and standard deviation unless otherwise stated. Two way ANOVA with a multiple comparisons test was used to investigate when differences arose in the GP results caused by both the amount of dilution and type of colloid. For the multi-comparison test as the number of comparison being used is large, only values of $P < 0.001$ are considered significant. Pearson correlation was undertaken to explore associations between d_f and standard markers of coagulation. Exact P values will be stated unless < 0.0001 .

Results

Rheometric Measurements: The Gel Point

The d_f at 0% dilution was 1.74 ± 0.033 , a result commensurate with the values reported for healthy volunteers in previous studies.^{13,14} For all three fluids an overall decrease in d_f is observed as dilution is increased (Figure 4a). Significant changes from the baseline (0% dilution) value were recorded at a dilution of: 20% for albumin (1.587 ± 0.056 , $P < 0.0001$); 40% for starch (1.561 ± 0.084 , $P < 0.0001$) and; 60% for gelatin (1.584 ± 0.061 , $P < 0.0001$). At 0% dilution the value of G'_{GP} was 0.0091 ± 0.0030 Pa. As the dilution was increased the strength of the incipient clot, as measured by G'_{GP} , decreases, which was consistent for all three fluids (see Figure 4b). The G'_{GP} is significantly reduced at a dilution of 20% from the baseline value for all three fluids ($P < 0.0001$ for albumin and starch and $P = 0.0009$ for gelatin). d_f and G'_{GP} were significantly correlated with each other ($r = 0.733$, $P = 0.0005$).

We used the two way ANOVA with a multiple comparisons test to determine whether there were any differences in the GP results when comparing the three fluids against each other. Whilst dilution did have a significant effect ($P < 0.0001$) on G'_{GP} no significant difference was observed between the three fluids ($P = 0.12$). For the d_f results we observe a significant difference due to dilution ($P < 0.0001$) but also due to the type of fluid ($p < 0.0001$).

Laboratory Markers

Blood was obtained from ($n = 104$) healthy volunteers. The results of the standard and specific laboratory markers for the three fluids are shown in Table 1. Hematocrit, fibrinogen, platelet count and thrombin generation all show a progressive linear

decrease as dilution is increased. Fibrinogen concentration and hematocrit show significant ($P < 0.001$) reductions from the baseline values occurring at a 20% dilution, or greater, for all three fluids. PT and APTT show a gradual increase in clotting time as dilution is increased for all three fluids. The PT and APTT were significantly ($P < 0.001$) prolonged at a 40% dilution or greater for all three fluids. When comparing the three fluids we found that there was no difference between them in terms of their standard laboratory markers.

We found significant correlations between d_f and the standard laboratory markers: fibrinogen ($r=0.588$, $p=0.0005$); Hematocrit ($r=0.652$, $p=0.0005$); platelet count ($r=0.457$, $p=0.0005$); PT ($r=-0.501$, $p=0.0005$); APTT ($r=-0.458$, $p=0.0005$); likely due to the dilution effect on all parameters.

Discussion

In this *in vitro* study of fixed volume dilution we quantify, using a novel viscoelastic technique, how the physiochemical properties of the three colloids exert individual changes on clot microstructure in addition to the effects of dilution. We found that of the three colloids tested gelatin caused the least changes in d_f compared to albumin which caused the largest changes ($P<0.0001$).

In a previous study, we quantified, using an isotonic crystalloid, how progressive dilution effects clot microstructure, producing a decrease in d_f as dilution increases.¹² In the present study we identified that three colloidal fluids also produce a steady decrease in d_f (Figure 4). However, Figure 4 illustrates that the magnitude of the changes on the d_f , is different depending on the type of colloid being used. We identified significant changes in d_f occurring at a dilution of 60% for gelatin ($d_f = 1.584 \pm 0.061$, $P<0.0001$), compared to both starch which occurred at a 40% dilution (1.561 ± 0.084 , $P<0.0001$) and albumin which occurred at a 20% dilution (1.587 ± 0.056 , $P<0.0001$). Analysis of the differences in d_f between the three fluids shows that there are significant differences between the three fluids that are independent of dilution ($p<0.0001$). This finding suggests that the properties of the three colloids themselves are altering coagulation. Additionally the response of coagulation (in terms of d_f) to *in vitro* dilution appears less impaired for gelatin than either the starch or albumin, and the most pronounced changes occurred with albumin (see Figure 4).

The intrinsic differences between the three colloids, reported by the GP parameters, was not reflected in the results of the standard coagulation markers (Table 1), no significant differences were observed. The standard laboratory markers seem to purely

measure the effect of dilution (see results of Hematocrit, fibrinogen and platelets) which all decrease by a similar magnitude with increasing dilution. The fact that the GP results identifies that the colloids do affect coagulation in different ways, suggests that the conventional markers cannot identify the intrinsic differences between them. Previous studies have highlighted that traditional coagulation screening tests, such as PT, APTT, platelet count and fibrinogen levels, are of limited value in acute hemorrhage and do not correlate well with bleeding outcomes which is confirmed in this study.²² This may be due to laboratory-based assays being carried out in platelet-poor plasma, omitting the vital contribution of the cellular aspects of coagulation. This is a major limitation as the intrinsic physiochemical properties of the different colloids are known to effect coagulation by interacting with platelets and other coagulation factors.²³⁻²⁷ Furthermore, different types of colloids will differ not only in what they affect but also in their severity.

The individual physiochemical properties of the colloids exert different intrinsic effects on coagulation and clot development. Many studies have shown that dilution with starch has a large detrimental effect on coagulation, and that this effect can be different depending on the type of starch used.^{28,29} Dilution with starch has been shown to: reduce platelet aggregation; cause an acquired von Willebrand syndrome; and affect fibrinolysis.^{27,30-32} Furthermore, *in vivo* studies link their use to increased bleeding and poorer outcome.³³ Whilst gelatin is known to inhibit platelet aggregation, it has been reported that its detrimental effect on coagulation is not as severe as either starch or albumin.^{4, 26,34}

In the present study of all the three fluids investigated, albumin produced the largest alterations in d_f . This is a somewhat controversial result considering it has been suggested that albumin is no worse than saline, having less effect on hemostasis than other synthetic colloids.³⁵ However, a previous *in vitro* study which investigated the effect of dilution with albumin compared to starch in whole blood, similar to the present study, has also shown that albumin caused the greatest derangement in clotting.⁷ Another study has also reported that albumin has a heparin-like effect on coagulation.³⁶ Possible explanations for this effect and the large changes observed in d_f may be due to the known inhibition of platelets by albumin, caused by binding to Platelet Activating Factor (PAF) and reducing histone mediated aggregation.^{24,25} Another important consideration in the change in d_f is that albumin has been shown to have an inhibitory property in fibrin polymerization, resulting in smaller diameter fibrils.³⁷⁻³⁸ Where d_f is a measure of clot microstructure and is directly affected by fibrin polymerization.

In this study we also utilize computer modelling to visually represent what the changes in the values of d_f mean in terms of clot mass and functionality. The values of d_f at higher dilutions, particularly for albumin, are noteworthy being very low (<1.5). The latter value has only been previously reported for blood samples anticoagulated with heparin at levels above the upper limit of the therapeutic range.¹³ Although values of d_f as low as 1.3 have been estimated for fibrin gel systems in light scattering studies,²¹ the latter were formed at markedly sub-physiological levels of fibrinogen ($<0.5\text{g/l}$). The mean fibrinogen concentration at 60% dilution with albumin in the present study being $0.9 \pm 0.2\text{g/l}$. Fractal systems for which d_f is less than 1.5 have relatively low levels of incorporated mass (Figure 3). Figure 3 shows that a clot that forms with a d_f of 1.50, will contain approximately 3-4% the mass of a clot formed with a d_f of 1.74 (0%

dilution). The example images of what typical fractal structures at d_f values of 1.74, 1.60 and 1.50 are shown in Figure 3. These images clearly represent how the changes in d_f and hence mass result in significantly different looking structures. The images show that structures with a higher d_f have a large amount of inter connectivity creating very strong clots. Consequently clots with lower values of d_f will have a low amount of inter connectivity and as a result will be mechanically weak and highly friable. These observations are supported by the very low values of G' associated with the higher dilutions in albumin (Figure 4). These systems may prove ineffective as microstructural templates for ensuing normal clot development, where such low levels of shear elasticity may be incompatible with haemostatic functionality with the clots bordering on being physiologically viable.

The study is the first to show how a marker of clot microstructure, in a preclinical model of dilution, can measure and differentiate between the intrinsic effects of different intravenous colloids. Although different fluids have similar and dissimilar intrinsic effects on the coagulation system, d_f was able to quantify the overall effect. The results from our previous¹⁶ and present studies, suggest that measurements of the GP may have clinically relevant implications in not only guiding the timing and amount of fluid required, but also the type of fluid and any additional blood products. The next phase of this research will include **investigating how particular coagulation factors and components modify the GP in hemodilution and assess GP various clinical settings to determine how changes in clot quality affect physiological parameters and outcomes.**

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Tables

	Dilution	Haemoglobin (g/l)	Hematocrit (l/l)	Platelet count ($\times 10^9$)	PT (sec)	APTT (sec)	Fibrinogen Conc. (g/l)
Baseline	0	13.8 (1.3)	0.409 (0.034)	271 (56)	10.5 (0.5)	25.6 (1.8)	2.6 (0.3)
Gelatin	10	12.8 (0.9)	0.374 (0.036)	229 (57)	11.2 (0.4)	29.3 (1.5)	2.3 (0.5)
	20	11.7 (0.9)	0.346 (0.025)*	194 (41)	11.9 (1.0)	31.1 (2.8)	1.8 (0.3)*
	40	8.6 (0.7)*	0.258 (0.018)*	140 (30)*	13.1 (1.1)*	36.7 (3.2)*	1.3 (0.4)*
	60	5.5 (0.6)*	0.164 (0.014)*	94 (19)*	18.1 (2.2)*	56.5 (7.5)*	0.8 (0.2)*
Starch	10	13.0 (1.3)	0.383 (0.025)	220 (35)	11.0 (0.6)	27.6 (0.9)	2.3 (0.2)
	20	11.1 (1.2)*	0.327 (0.034)*	217 (24)	11.3 (0.5)	29.7 (1.6)	2.0 (0.2)*
	40	8.3 (0.8)*	0.253 (0.023)*	157 (44)*	12.8 (1.0)*	35.1 (3.1)*	1.4 (0.4)*
	60	5.7 (0.5)*	0.169 (0.014)*	95 (25)*	16.8 (1.9)*	54.9 (9.6)*	1.0 (0.1)*
Albumin	10	12.6 (1.1)	0.378 (0.029)	219(36)	10.7 (0.3)	26.5 (0.9)	2.3 (0.4)
	20	11.1 (1.0)*	0.333 (0.023)*	192 (41)	11.2 (0.2)	28.0 (1.2)	2.2 (0.7)*
	40	8.5 (0.8)*	0.247 (0.019)*	145(25)*	12.9 (0.5)*	33.1 (1.7)*	1.4 (0.4)*
	60	5.7 (0.5)*	0.172 (0.013)*	94 (17)*	16.7 (1.0)*	51.2 (5.9)*	0.9 (0.2)*

Table 1: Table of results for the standard laboratory markers for all three fluids at all dilutions. (n=8 for each dilution) *denotes a significant deviation from the baseline value using two way ANOVA multiple comparisons test analysis where $p < 0.001$.

Figure Legends

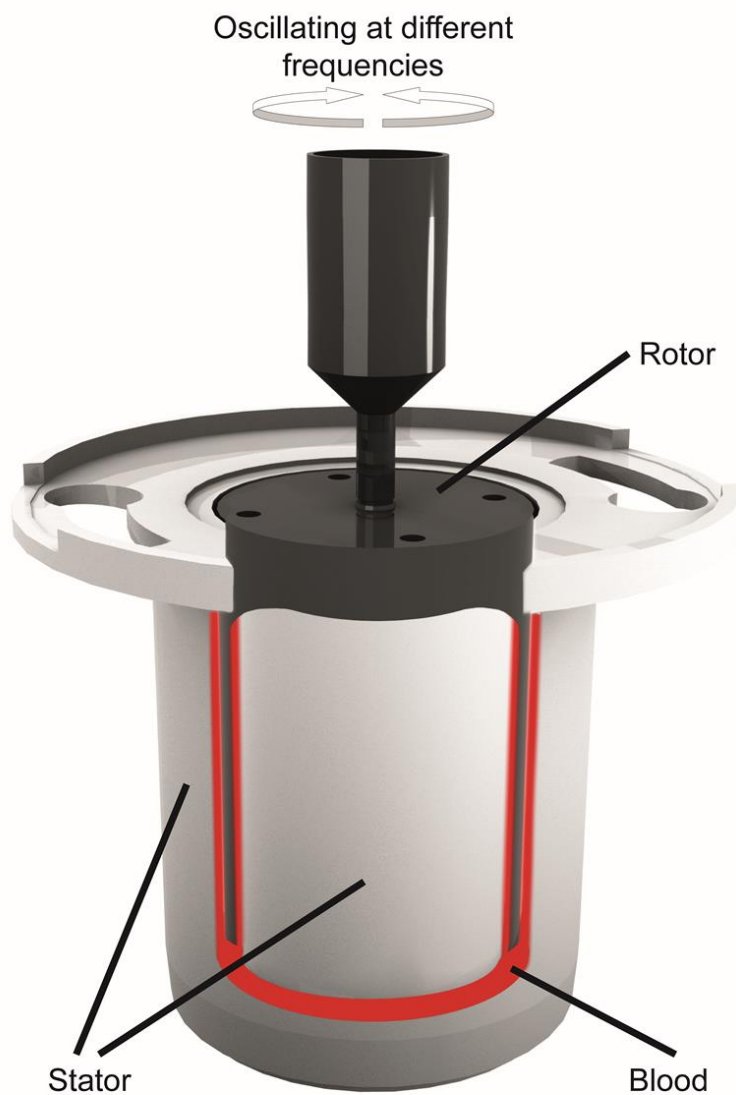


Figure 1 – Diagram of a Double Gap Concentric Cylinder Measurement Geometry. The double gap geometry consists of a stationary cup or stator into which a 6.6ml sample of blood is added. After which a bob which is free to rotate, called a rotor is then lowered into the sample. The movement of the rotor is controlled by an AR-G2 Controlled Stress Rheometer and will oscillate at 4 different frequencies (0.20Hz, 0.43Hz, 0.93Hz and 2.00Hz) sequentially over time.

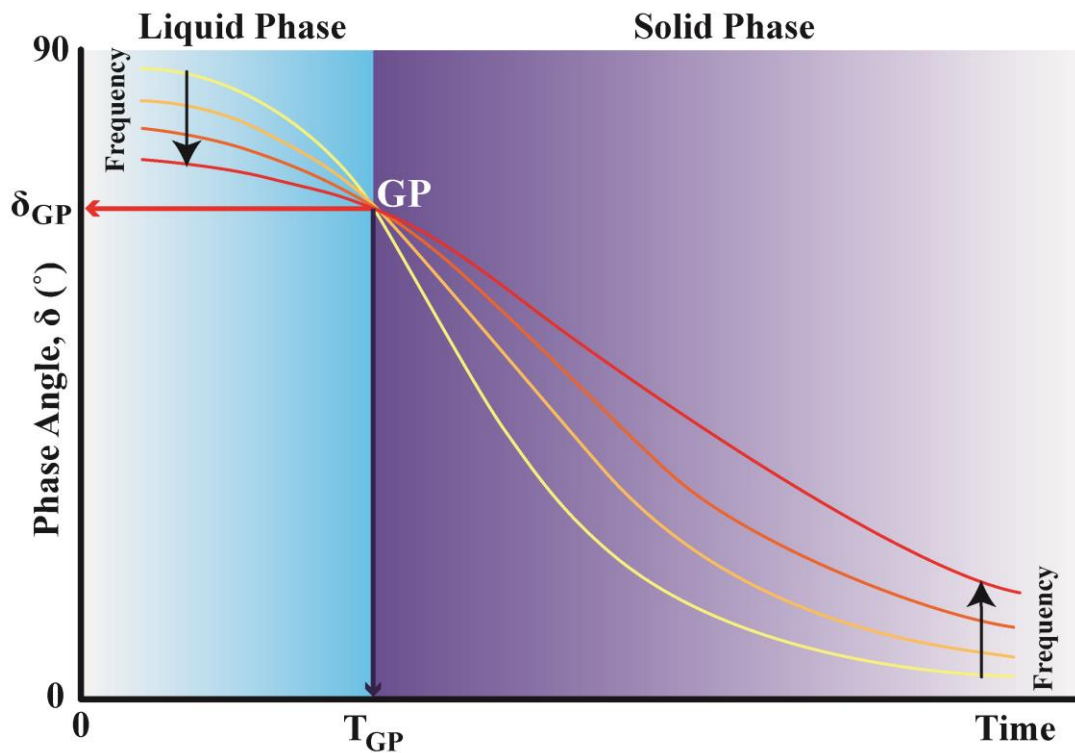


Figure 1

Figure 2: Gel Point Trace: This represents a typical GP result for one sample of blood. The illustration demonstrates how phase angle, δ , changes as coagulation progresses. δ has a range of 0 to 90° where 90° identifies a purely viscous response and 0° identifies a purely elastic response, with any value in between being a measure of the viscoelastic response to imposed stress. In a material that is changing from a liquid to a solid, such as blood, there will be a decrease in δ . At the establishment of the incipient clot, when the clot becomes a viscoelastic solid, there is a point where the value of δ will be independent of frequency, called the GP. The structural property of the incipient clot (in terms of its fractal dimension, d_f) is derived from the frequency independent value of δ_{GP} .

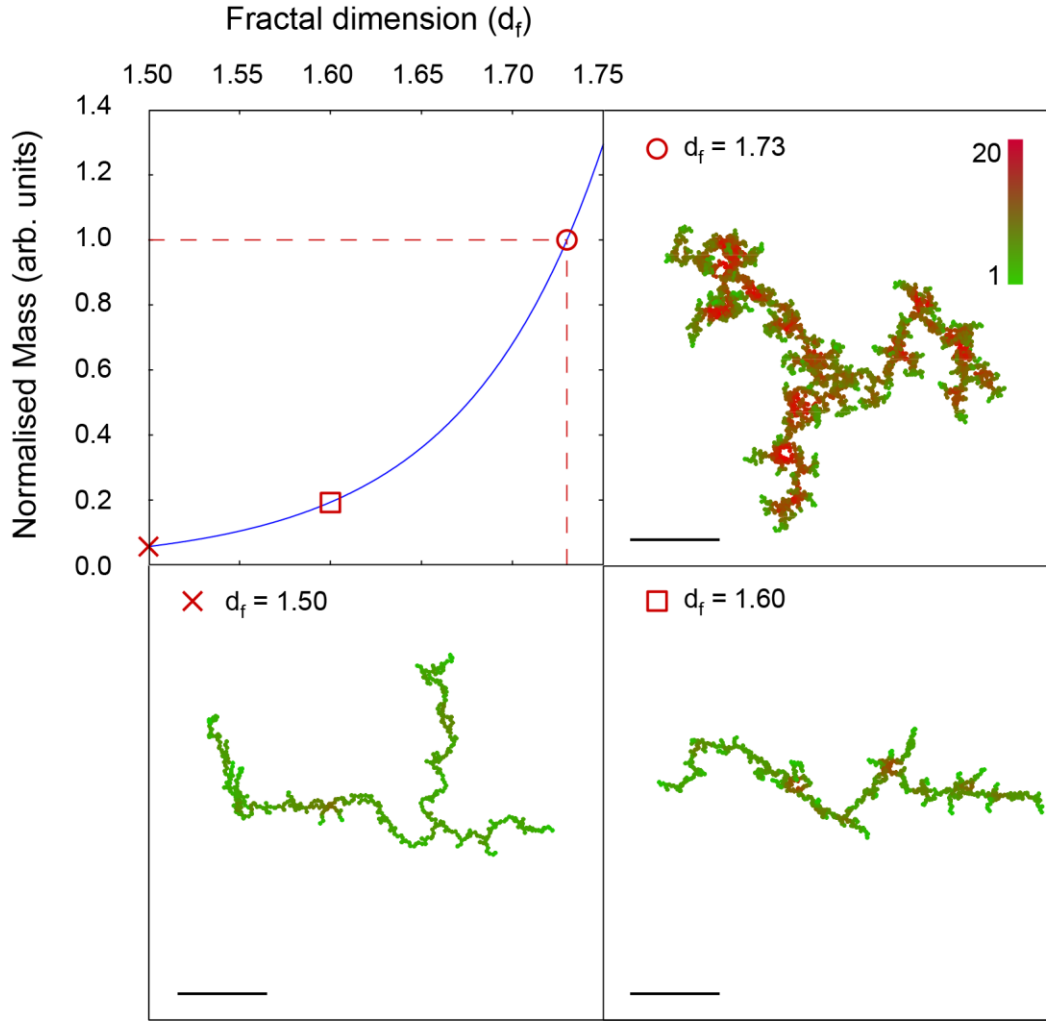


Figure 3 – Graph of fractal dimension, d_f vs. Mass. Illustration of the non-linear relationship between d_f and the amount of mass incorporated into the structure. The mass value on the y-axis is normalized for the healthy index value of d_f ($=1.74$). Illustrations of different incipient clot microstructures that correspond to particular values of d_f are provided (cross = 1.50, circle = 1.73 and square = 1.60 respectively). The color of each node (unit sphere) within the fractal represents the local density of constituent nodes within a sphere of radii 5 units, the color ranges from green (1 neighboring node) to red (20 neighboring nodes). The high density of red (nodes) in the $d_f = 1.74$ images shows a large amount of inter connectivity creating a very strong clot. As d_f is reduced the number of these dense areas (red) will reduce where at 1.60 there are only a handful of red areas and at 1.50 there are no deep red areas.

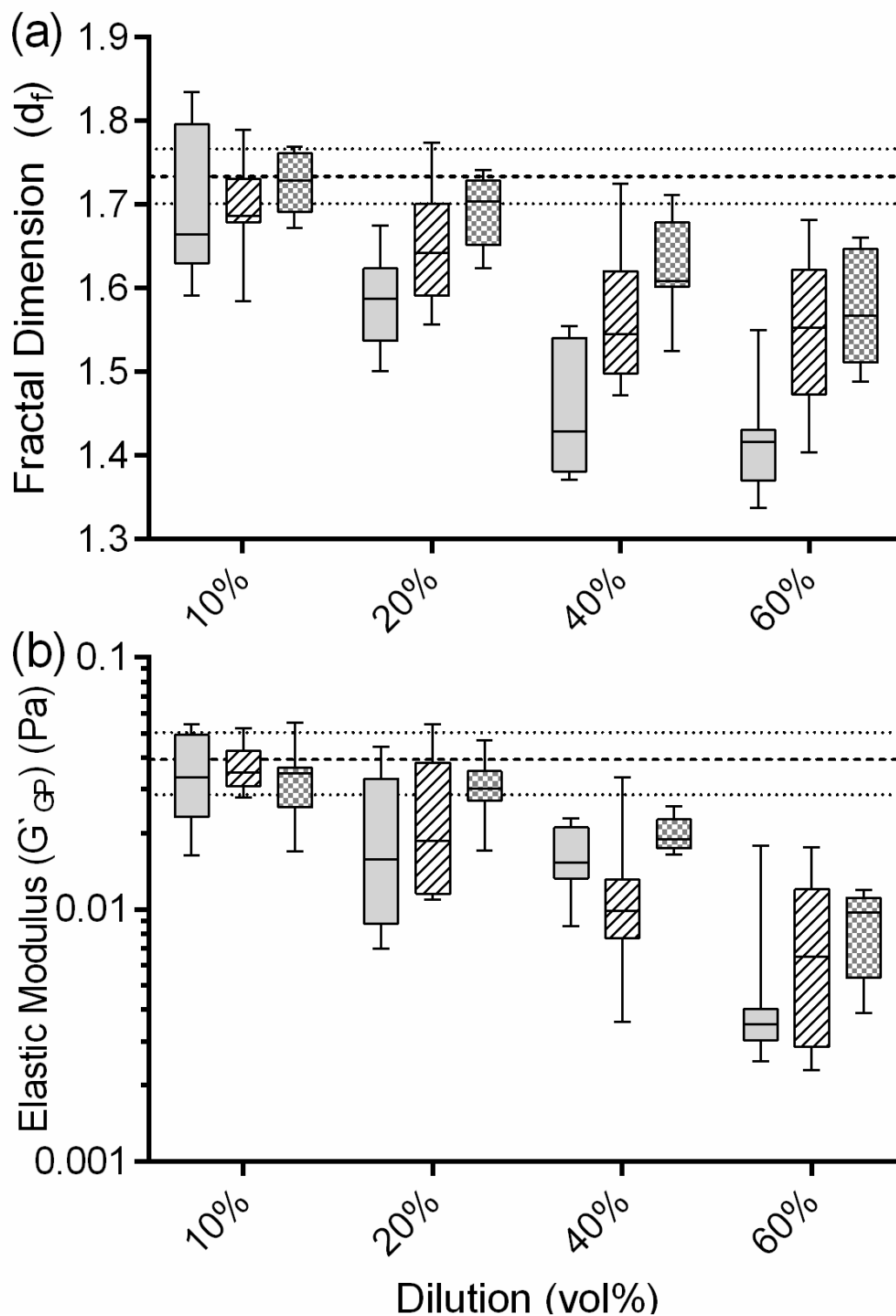


Figure 4: Graph of the Gel Point parameters: Box and whisker plot illustrating the change in d_f & G'_{GP} for the three different colloids for the 4 dilutions, 10%, 20%, 40% and 60%.